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(54) Abstract Title
Multiwell plate

(57) A multiwell flat plate useful in combinatorial chemistry for controlling the distribution of beads on particles comprises a first side with an array of wells of circular cross section and circular openings where each well is connected to a hole which passes to the second side of the plate wherein each hole has a circular opening in the well to which it is connected and the opening of each hole in each well is smaller than the opening of the well on the first side of the plate. A suspension of beads in a liquid may be applied to the first side of the plate and a pressure difference applied to hold one bead per well.

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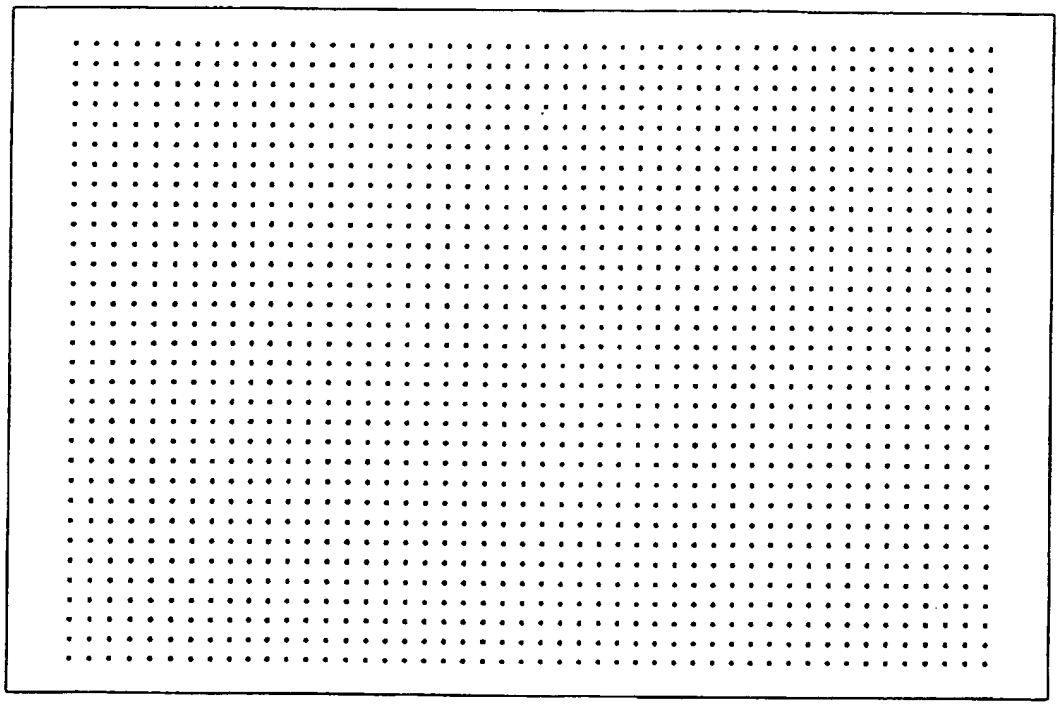

FIG. 1

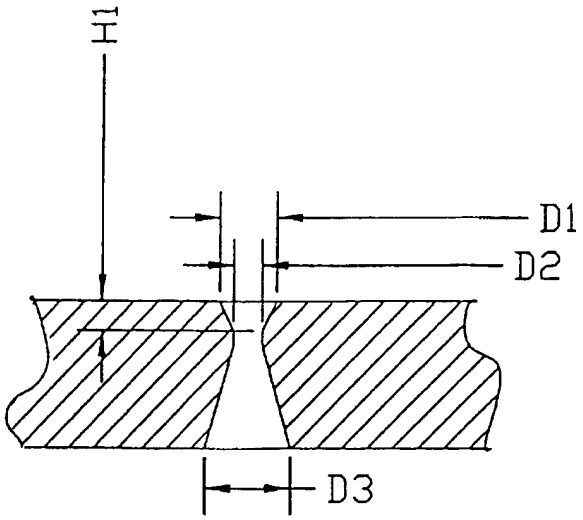

FIG.2

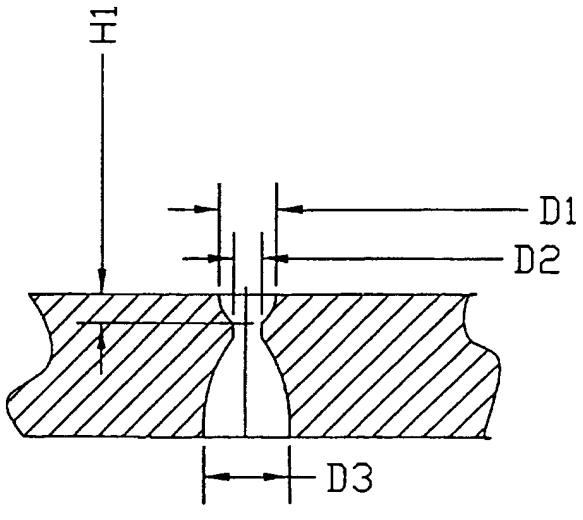

FIG.3

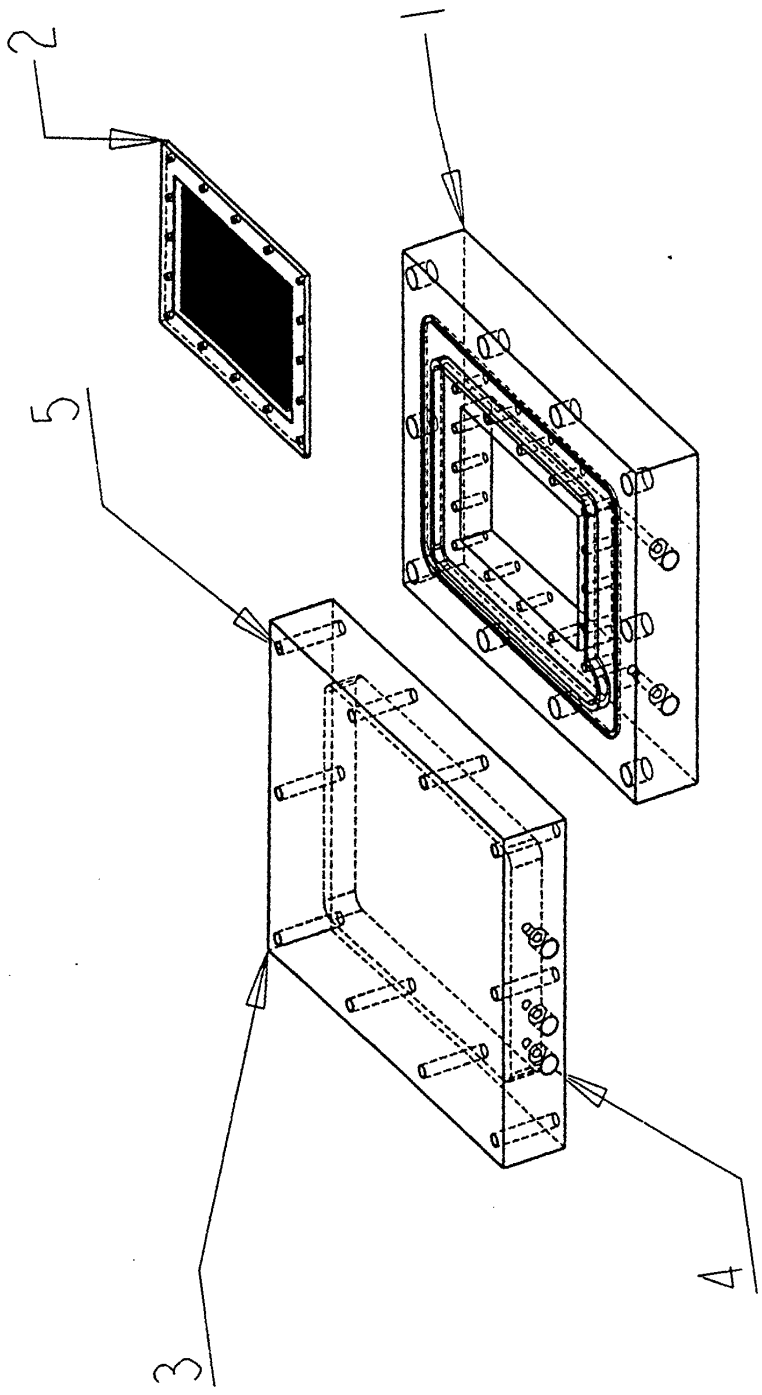

FIG.4

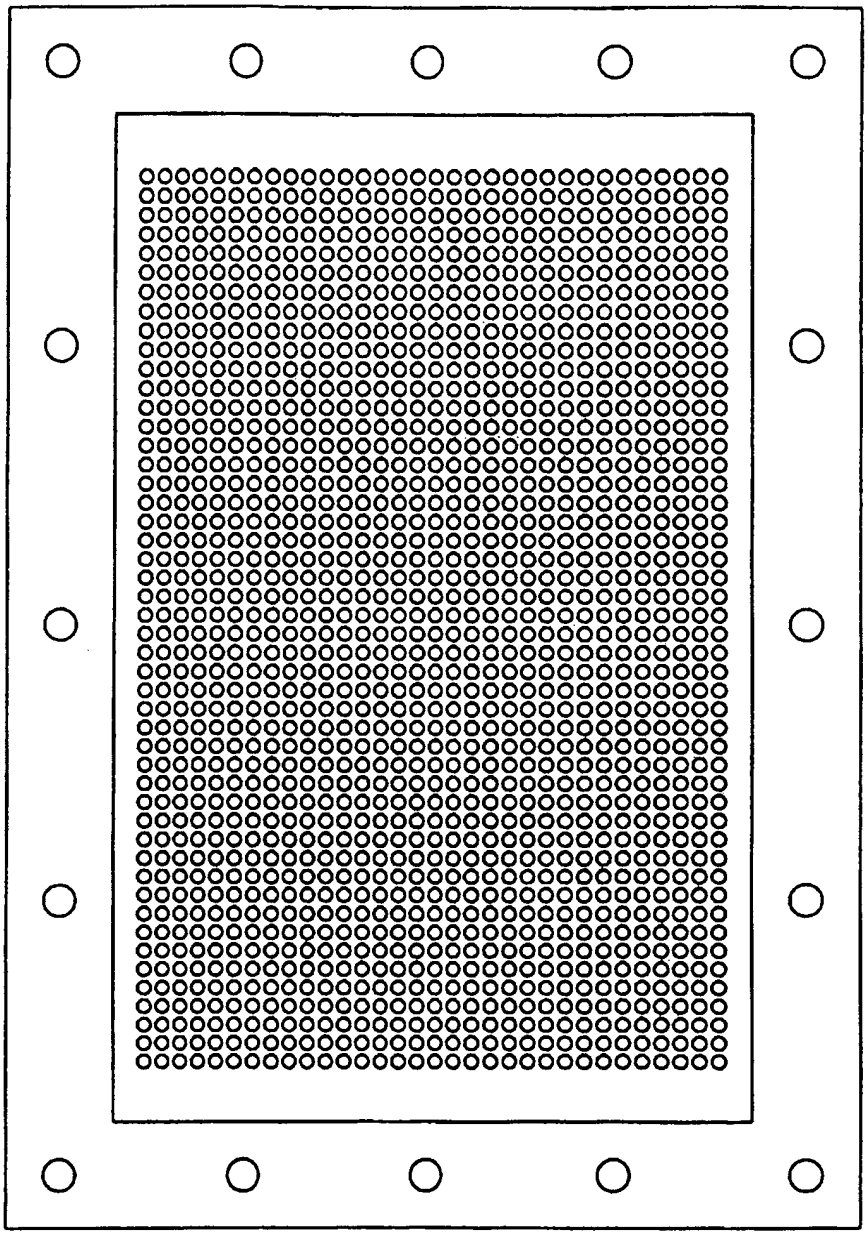

FIG.5

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TITLE OF THE INVENTION
METHOD AND APPARATUS FOR CONTROLLED DISTRIBUTION OF SUPPORTS IN COMBINATORIAL SYNTHESIS

FIELD OF THE INVENTION

This invention relates generally to the field of combinatorial chemistry, and more specifically to methods and apparatus for uniformly controlling the distribution of beads or particles of a combinatorial support on a plate or other plate.

BACKGROUND OF THE INVENTION

The standard method for searching for new chemical compounds which can effectively modulate biological processes employs the screening of pre-existing compounds in assays which have been designed to test particular properties of the compound being screened. Similarly, in designing compounds having desired physiochemical properties for general chemical applications, numerous compounds must be individually prepared and tested.

To reduce the time and expense involved in preparing and screening a large number of compounds for biological activity or for desirable physiochemical properties, technology has been developed for providing libraries of compounds for the discovery of lead compounds. Current methods for generating large numbers of molecularly diverse compounds focus on the use of solid phase synthesis. The generation of combinatorial libraries of chemical compounds by employing solid phase synthesis is well known in the art. For example, Geysen, et al. (Proc. Natl. Acac. Sci. USA, 3998 (1984) describe the construction of multi-amino acid peptide libraries; Houghton, et al. (Nature, 354, 84 (1991) and PCT Patent Pub. No. WO 92/09300) describe the generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery; Lam, et al. (Nature, 354, 82 (1991) and PCT Patent Pub. No. WO 92/00091) describe a method of synthesis of linear peptides on a solid support such as polystyrene or polyacrylamide resin.

The growing importance of combinatorial chemistry as an integral component of the drug discovery process has spurred extensive technological and synthetic advances in the field. See Thompson, L. A., and Ellman, J. A., *Chem. Rev.* **96**, 555-600 (1996)). Founded in peptide synthesis devised by Merrifield, solid phase chemistry has emerged as the preeminent method for construction of small molecule combinatorial libraries. See for example: (a) Merrifield, R .B., *J. Am. Chem. Soc.* **85**, 2149-2154 (1963); (b) Terrett, N. K.; Gardner, M.; Gordon, D. W.; Kobylecki, R. J.; and Steele, J., *Tetrahedron* **51**(30), 8135-8173 (1995); and (c) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; and (d) Gallop, M. A., *J. Med. Chem.* **37**, 1385-1401 (1994).

It is known that a wide variety of organic reactions can be carried out on substrates immobilized on resins. These include, in addition to peptide synthesis, nucleophilic displacements on benzylic halides, halogenation, nitration, sulfonation, oxidation, hydrolysis, acid chloride formation, Friedel-Crafts reactions, reductions, metallation, and the like which are well known in the art. (See for example, Mathur, et al., "Polymers as Aids in Organic Chemistry", Academic Press, New York, 18 (1980) and Farrall, et al., J. Org. Chem., 41, 3877 (1976)).

Current methods of drug discovery often involve assessing the biological activity (*i.e.*, screening) of tens of thousands of compounds in order to identify a small number of those compounds having a desired activity. The assays are generally carried out in multi-well tissue culture plates called microtiter plates. Microtiter plates are usually made of plastic, with the wells being formed by indentations in the bottom of the microtiter plate. For screening, commonly used microtiter plates have 96 individual wells, although the trend is to use higher density plates of 384, 864, 1536 , 3456, and even 9600 wells. The creation and screening of combinatorial libraries is discussed, for example, by Brown in Molecular Diversity, 2, 217-222 (1996).

Split-pool synthesis on a solid support is a powerful way of making combinatorial libraries. Often thousands or hundreds of thousands of compounds can be synthesized in a short time and at low cost; for example, see Furka, A., et al. "General Method for Rapid Synthesis of Multicompounent Peptide Mixtures", Int. J. Protein

Peptide Res, 37, 487-493 (1991). Although each individual solid support particle carries only one member of the library, mixtures of such solid support particles are produced at the end of the synthesis process. Compounds can be cleaved free from the solid support, giving a set of samples in the form of a mixture for biological assay. After completion of the assay, the active individual compounds are determined based on the results obtained with mixtures of free or supported compounds. The most potent compound(s) within the library are determined using an iterative deconvolution process based on the feedback from the mixture assay results. Such a process is tedious, and there is no guarantee that the best compounds in an active mixture can be determined by using an iterative deconvolution process. Furthermore, not all biological assays are fit for mixtures rather than single component samples. Alternative methods of carrying out iterative deconvolution have been taught in the literature, but these are not always successful either.

To avoid the need for iterative deconvolution, the solid support particles may be kept spatially separated through the entire assay process. Biological assay is then carried out around each individual solid support particle without releasing the compound being assayed from the solid support particle (see S. L. Schreiber in published PCT patent application WO 98/16830). The structure of the compound on particles of support that are active is readily determined.

Alternatively, the compound may be cleaved from the spatially separated individual solid support particles to give a single sample of free compound. This makes it possible to carry out a wider range of biological assays, since the compound is no longer bound to the support.

It would be highly desirable to have a method for achieving a controlled distribution of a combinatorial support, in particular a solid support such as a resin bead support, on a plate. This would facilitate cleavage of the compounds from the support and subsequent transfer onto microtiter plates so that screening of the compounds derived from the supports can be carried out on the microtiter plate.

Methods for spreading beads as a high density array on a plate have been described in the literature. One example involves

suspending resin beads in agarose gel and then applying the suspension to an array of wells (Anal. Biochem., 246, 20-29 (1997)). This results in a statistical distribution of beads in wells, some of which are empty, others having multiple beads. Reagent is wasted in the empty wells, resulting in a lower assay density, whereas wells containing multiple beads complicate the assay and make identification of active compounds much more difficult. Another known method involves spraying beads suspended in a liquid through a fine nozzle onto a plate, but this provides little control of the bead distribution on the plate. A variation of this last approach is to sprinkle the beads in suspension onto a thin polymer sheet and then to stretch the sheet to spread out the resin beads (Proc. Natl. Acad. Sci. USA, 91, 1614-1618 (1994)). This method also provides only limited control of bead distribution, with aggregation of beads in one place often occurring. The present invention addresses the numerous disadvantages of these earlier methods that are described above.

SUMMARY OF THE INVENTION

The present invention provides a method for distributing combinatorial support particles in a 2-dimensional array on a flat plate, by using the following steps:

(a) providing: (1) a suspension of combinatorial support particles in a liquid; and (2) a flat plate having a first side and a second side and an array of wells on the first side of the plate, where each well has an opening on the first side of the plate, where each well is also connected to a hole that passes from the well to the second side of the plate, and where each hole has an opening in the well to which it is connected; where the diameters of substantially all of the combinatorial support particles are larger than the diameters of the openings of the holes in the wells;

(b) applying the suspension of the particles to the first side of the plate;

(c) creating a positive pressure difference between the first side and the second side of the plate by applying a vacuum to the second side of the plate, or a positive pressure to the first side of the plate, or a combination of a vacuum and a positive pressure to the second and first

sides of the plate respectively, so that the particles are held in or on the wells by the pressure difference between the first side and the second side of the plate, with one particle being held on or in each well; and

(d) washing the excess particles from the plate with a liquid, so that the particles of support that remain after washing are held on or in the wells, with one particle per well. By following this method, the particles are distributed on the plate according to the pattern of the holes on the plate, and are separated from each other according to the spacing of the holes on the plate.

Preferably, the particles are on the openings of the wells rather than in the wells, with the diameter of the particles being larger than the diameters of the openings of the wells. However, because it is very difficult to obtain particles that are all the same size, some of the smaller particles may fall into the well. The opening of the hole inside the well is smaller than the opening of the well, so that preferably even the smaller particles will be too large to pass through the hole that leads to the other side of the plate.

The preferred kinds of combinatorial support particles are combinatorial support beads, which can be purchased commercially for many applications, or custom synthesized, if necessary. The invention thus is also a method of distributing combinatorial support beads in a 2-dimensional array on a flat plate, according to the following steps:

(a) providing: (1) a suspension of combinatorial support beads in a liquid, where the beads are approximately spherical in shape and have an average diameter in the range of about 50 microns to about 5,000 microns; and (2) a flat plate having a first side and a second side and an array of wells on the first side, where each well has an approximately circular cross section in the plane of the plate, with an approximately circular opening on the first side of the plate, where each well is connected to a hole that passes from the well to the second side of the plate, each hole having an approximately circular opening in the well to which it is connected, where the opening of each hole in the well to which it is connected is smaller than the opening of the top of the well (on the first side of the plate) and is smaller than substantially all of the beads ;

(b) applying the suspension of beads to the first side of the plate;

(c) creating a positive pressure difference between the first side and the second side of the plate by applying a vacuum to the second side of the plate, or a positive pressure to the first side of the plate, or a combination of a vacuum and a positive pressure to the second and first sides of the plate respectively, so that the beads are held on the openings of the wells by the pressure difference between the first side and the second side of the plate, with one bead being held on the opening of each well; and

(d) washing the excess beads from the plate with a liquid, so that the particles of support that remain after washing are held on the openings of the wells, with one particle per well.

The invention also is a method for distributing combinatorial support particles or beads in a 2-dimensional array on a flat plate that has holes passing through it but does not have discrete wells on the first (upper) side, as described above. In this situation, the method comprises the following steps:

(a) providing: (1) a suspension of combinatorial support particles, which typically are beads, in a liquid; and (2) a flat plate having a first side and a second side and an array of holes that pass from the first side to the second side of the plate, wherein the holes have openings on the first side and second side of the plate, where the holes on the first side of the plate are smaller than the average diameter of the support particles;

(b) applying the suspension of particles or beads to the first side of the plate;

(c) creating a positive pressure difference between the first side and the second side of the plate by applying a vacuum to the second side of the plate, or a positive pressure to the first side of the plate, or a combination of a vacuum and a positive pressure to the second and first sides of the plate respectively, so that the particles or beads are held on the holes by the pressure difference between the first side and the second side of the plate, with one particle or bead being held on the opening of each hole on the first side of the plate; and

(d) washing the excess particles or beads from the plate with a liquid, so that the particles or beads that remain after washing are held on the openings of the holes on the first side of the plate, with one particle or bead per hole.

An additional aspect of the present invention is directed to the plate for capturing individual combinatorial support particles or beads in an array, where the particles or beads are distributed on the plate with essentially one bead per well. The particles or beads are preferably spatially separated to facilitate compound cleavage, identification, and assaying on each individual particle or bead.

The plate used in this process has a first side, a second side, and an array of wells on the first side, with each well having an approximately circular cross section in the plane of the plate and an approximately circular opening on the first (upper) side of the plate, with each well being connected to a hole that passes from the well to the second side of the plate. Each hole has an approximately circular opening in the well to which it is connected, and the opening of each hole in each well is smaller than the opening of the well on the first (upper) side of the plate. In preferred embodiments, the diameters of the well openings on the first side of the plate are in the range of about 40 microns to about 4000 microns and the thickness of the plate is in the range of about 0.1 mm to about 5 mm. In more preferred embodiments, the openings of the wells are in the range of about 80 microns to about 1,600 microns.

The combined apparatus for distributing combinatorial support particles on a plate is also new. The apparatus needed for carrying out the process includes the plate described above and the means for creating a pressure differential between the first side of the plate and the second side of the plate. This is preferably a manifold which holds the plate and supports it from underneath and is designed to permit the application of a vacuum under the plate, application of a pressure higher than atmospheric pressure above the plate, or a combination of both. The manifold also has provision for the addition and removal of liquids and the admission of gases, which may be reactive vapors.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a top view of a high density combinatorial support plate having 1,536 holes.

Figures 2 and 3 are side views of the wells and holes, illustrating two different shape profiles.

Figure 4 is a disassembled view of the manifold that was used to handle the plates.

Figure 5 is a top view of the stainless steel plate (2 in Figure 4) that is used to hold the combinatorial support plate.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides an apparatus and methods for the controlled distribution of particles of a solid support which are preferably in the form of beads that are approximately spherical in shape and that all have approximately the same diameter, into a 2-dimensional array on a plate. The support beads are often made of a polymeric resin, such as polystyrene or polyacrylamide.

The particles or beads are distributed onto an array of wells or onto an array of holes, with a distribution of one bead per well or hole. Preferably, the holes or wells are arranged with the same distribution and spacings as are found in microtiter plates that are used by practitioners in the field of combinatorial chemistry, so that the plates may be used with automated equipment currently in use in the field of combinatorial chemistry. The wells or holes may also be arranged in one of the high density formats that are commonly in use in the field. Using this method, combinatorial libraries made in split-pool fashion can rapidly be screened as single compounds, with each bead/compound being spatially fixed on the plate. The present invention may readily be employed as a component of a high throughput system for screening a combinatorial library in single-compound format, and thus facilitates the integration of combinatorial synthesis with high throughput screening.

In a preferred embodiment of the invention, a high density array of wells is fabricated onto a plate. A hole is present at the bottom of

each well, or at least at the bottom of substantially all of the wells. Alternatively, there may not be wells at all, but only an array of holes that pass entirely through the plate.

The wells in general are designed to act as holders for combinatorial support particles. The wells preferably have a circular cross section in the plane of the plate, and preferably have a circular opening at the top of the well, with the diameter of the well decreasing with distance from the top of the well. The wells are sized to hold one support bead each. The combinatorial support particles have a diameter in the range of about 50 microns to about 5000 microns, more typically about 100 microns to about 2,000 microns.

Typical profiles of wells and holes are shown in Figures 2 and 3. Both figures illustrate a well with a hole in the bottom that passes through the plate from the bottom of the well. The wells are larger at the top, and the holes may also be flared as they exit the bottom of the plate. There is a narrow point (i.e. a constriction) in the overall passageway between the two sides of the plate at the point where the hole opens into the well.

This design often gives the combination of the well and hole an hourglass appearance. In Figures 2 and 3, D1 is the diameter of the opening of the top of the well on the first side of the plate. D2 is the diameter of the constriction between the bottom of the well and the hole passing through the plate, and is referred to as the diameter of the opening of the hole at the bottom of the well. The diameter of the hole at the bottom of the plate is the distance D3 in Figures 2 and 3. Typically D1 is smaller than the average diameter of the combinatorial support particles, so that the particles rest on the opening of the well rather than inside the well. D2 is chosen to be smaller than the diameter of all or substantially all of the combinatorial support particles, so that the particles do not block or pass through the holes. D2 is preferably about 40% to 60% of D1, though D2 may be somewhat larger than the preferred range or much smaller. The openings at the bottoms of the holes on the second side of the plate are often larger than the inner diameters of the holes. This last diameter (D3) is fairly unimportant compared with D1 and D2. D3 must be large enough to permit application of vacuum to the

bottom of the plate and to allow the application of air pressure or vacuum for collection of the liquid from the combinatorial supports. The diameter D3 may often be in the range of about half the diameter of the opening of the well (D1) to double the diameter of the opening of the well at the top of the plate. Typical dimensions of D1 are about 40 microns to about 4000 microns, preferably about 80 microns to about 1,600 microns, and most preferably about 250 microns to about 400 microns.

A drawing of the top view of a high density plate is shown in Figure 1. The dimensions of the rectangular plate are 120 mm x 78 mm, and the thickness is 0.5 mm. The plate illustrated has a standard array of 1,536 holes (32 rows x 48 columns), with a spacing between holes of 2.25 mm. Other high density formats can of course also be used, including but not limited to, high density formats currently in use having arrays of 96, 384, 864, 1536, 3456, and 9600 wells per plate.

In an alternative embodiment to the above design using holes and wells, the plates could simply have holes passing through from the top to the bottom, without the presence of a distinct well and hole as was described above. The hole would then most likely be approximately cylindrical, with perhaps some flaring of the opening at the top and/or bottom. The dimensions of the openings of the holes would be the same as described above for the openings of the wells compared with the bead diameter (i.e., the diameter of the openings of the wells or holes on the first side of the plate, which is where the beads rest, is smaller than the diameter of substantially all of the beads, preferably is smaller than the diameter of the beads down to about 10% of the diameter of the beads, and most preferably about 80% to about 50% of the diameter of the beads).

Particles of solid support material are typically used in the form of polymeric beads or beads made of other materials. The beads are approximately spherical in shape and have a diameter greater than the diameter of the openings of the wells and of the openings of the holes in the bottoms of the wells, or of the holes that go through the plate in the cases where there are no wells. The bead diameters may be up to about 10 times the diameter of the openings at the top of the wells or of the openings of the holes and typically are about 1.25 to about double the

diameters of the openings of the wells or holes. Since the beads are spherical and often fragile, it is preferred that the holes and wells are round where they are in contact with the beads to prevent breaking of the beads. It is most convenient, though not absolutely necessary, that the holes and wells have a round cross-section all the way through the plate, since a hole or well with a circular cross-section is generally easier to make and presents a round opening no matter where the bead fits in the well or hole.

The support particles are most often made of a polymeric resin that has been chemically functionalized so that the compounds comprising the library can readily be attached and removed from the particle. Other types of materials, such as glass, can also be chemically functionalized. Typical polymeric resins used as beads include polystyrene, polyethylene glycol-grafted polystyrene, and polyacrylamide. Such chemically modified polymeric or other beads, methods of attaching and synthesizing compounds on the beads, and methods of detaching compounds from the beads are all well known in the art.

In order to distribute the beads on the plate, the beads are first suspended in a liquid that is a non-solvent for the beads (for example, DMF is a suitable liquid in the case of polystyrene beads), and the suspension is applied to the top of the plate. A partial vacuum is applied under the plate, for example at approximately 5-15 psi vacuum, or an elevated pressure is applied above the plate, or a combination of both vacuum and pressure are applied. This removes the liquid and holds the beads against the openings of the wells. Excess beads are then removed by washing the plate with the liquid in which the beads were suspended. A controlled distribution of one bead per well is achieved, where one bead resides at the top of each well. This process avoids problems that occur in other known processes where the distribution process is more random in nature. These problems inherent in more random processes include the presence of many wells that are empty and many other wells that have multiple support particles.

Because the size of the beads is larger than the diameter of the openings of the wells, the beads do not generally go into the wells,

but are instead held loosely to the tops of the wells by the pressure difference that is created by the partial vacuum between the top and bottom of the well. The pressure differential between the top and bottom of the plate may also be created by application of a positive pressure to the top of the plate, or a combination of a positive pressure at the top of the plate and a partial or full vacuum at the bottom of the plate. This results in a controlled distribution of beads, with an ideal distribution of one bead per hole. There may be minor deviations from the ideal distribution due to beads not falling into place in every hole. However, in general at least 95%, and preferably at least 99%, of the holes will have one and only one bead, with the remainder of the holes generally having no beads.

An apparatus that can be used to apply a vacuum or partial pressure to the plates is shown in Figures 4 and 5. Figure 4 illustrates a manifold for holding and supporting a combinatorial plate while performing manipulations on the beads, such as rinsing, by applying a pressure and/or vacuum to hold the beads in place. Figure 4 also illustrates a stainless steel sheet for holding the combinatorial support plate. The bottom piece of the manifold is marked 1 in Figure 4, and the top piece is shown as 3. Both of these pieces have length and width dimensions of 240 mm x 190.5 mm, with a height of 38.1 mm. The combinatorial support plate is supported on 2 in Figure 4, which is a 316 stainless steel sheet (3.175 mm thick) with indentations to hold the combinatorial support plate in place. The sheet has 1.5875 mm holes that match the holes in the combinatorial support plate, so that the stainless steel sheet does not interfere with any of the processes (filtration, vacuum, etc) being carried out on the combinatorial support plate. The top and bottom pieces of the manifold 1 and 3 have indentations to hold the stainless steel sheet 2, which in turn holds the combinatorial support plate. The whole apparatus is held together with bolts that pass through vertical holes like the one that is marked as 5 in Figure 4. In addition there are openings 4 in one side of both 1 and 3 for the introduction of vacuum under the plate and pressure above the plate, as well as the introduction or removal of liquids or chemical vapors (e.g.

trifluoroacetic acid). The openings 4 can accommodate hose connectors for attachment of flexible tubing.

Once the beads are distributed in this well-controlled format by using the device described above, their biological activities may be assayed by methods well known in the art. On-bead assays can be carried out by adding assay media directly to the beads of solid support, which may be held in the array by continuing application of partial vacuum and/or pressure.

This method of distributing polymer beads is more broadly useful when the compounds that are bound to the resin are released from the beads into liquid drops that are confined to the area surrounding the beads from which the compounds were released. Then solution methods for performing assays, chemical analysis and the like, may be used. Support-bound chemical compounds may be cleaved via conventional cleavage methods, such as photochemical cleavage, acid/base effected cleavage, or other methods which are well known in the art. Photochemical cleavage is the preferred method of cleavage. The compounds after cleavage are in a drop of liquid around the bead that formerly supported it, either in a well or on a support plate with holes. The use of a well is generally more convenient.

Once the cleavage reactions have been completed, the combinatorial library which originally had been made in split-pool fashion is transformed from a mixture of compound-carrying supports into a library of single compounds, each in solution. This collection of compounds may be screened or partitioned multiple times for subsequent biological screens and/or chemical identification. Assays can now be carried out on single compounds rather than on mixtures of compounds. Thus, a wide range of biological assays (membrane assay, cell assay, etc.) can now be run on single compounds. Coupled with encoding/decoding techniques, the library is screened without the necessity of iterative deconvolution, yielding more complete information about the library in a much shorter period of time.

The plates employed in the present invention can be made by a variety of techniques and from a variety of materials, some of which are explained below.

The plate may be made from silicon, glass or other etchable materials. In particular embodiments, the material used to make the plate is typically selected from the group consisting of plain glass, derivatized glass, silanized glass, glass having absorbed bio- and non-biopolymers, polystyrene, and other plastics, indium tin oxide and other metal oxides, gold and other metals, and ceramics. Alumina is a preferred ceramic. Derivatized glass is glass that has had its surface chemically modified to something other than only SiO_2.

The plate may be prepared by etching a thin planar material such as glass, silicon, metal, or ceramic with an anisotropic etch through to a stop to get a hemispherical indentation (without a top) once the etch stop is removed. A hydrophobic material such as a fluoropolymer (e.g. TEFLON®) may be applied by silk-screening to form the wells. Alternatively, an etchable material, such as, glass, silicon, metal or ceramic may be etched with an isotropic wet etch, plasma etch, reactive ion etch, or bombardment technique from one side or with either an isotropic or anisotropic etch from both sides to form an hourglass type hole or a linear or conical shaped hole. Alternatively, the material may be laser drilled from one or both sides to form straight, conical or complex shaped holes. Likewise, high pressure water drilling may be employed to prepare the holes. Similarly, a laminating technique may be employed to layer on ceramic or other material to form a shaped hole. In any of the above methods, a non-noble metal may be applied and then coated with gold or platinum before a fluoropolymer is applied by silk-screening. Standard machining with a small drill bit may also be employed to prepare the wells. An array of cones or pyramids may be pressed into glass, metal or ceramic substrate by heating the cones, pyramids, or substrate. Alternatively, a molten or liquid material (such as spin glass, glass, or plastic) may be poured onto a form, or a softened material may be pressed into a form to make the holes. In other cases, a chemically inert, relatively hydrophilic, plastic may be employed to form the capture plate by injection molding, thermoforming, or other traditional plastic molding techniques. The method that is currently preferred for making the plates is drilling holes in a plate made of alumina using a a high powered laser.

A "combinatorial library" is a collection of compounds in which the compounds comprising the collection are composed of one or more subunits or monomeric units (i.e. synthons). The subunits may be selected form natural or unnatural moieties including amino acids, nucleotides, sugars, lipids, carbohydrates, dienes, dienopholes, and the like. The compounds of the combinatorial library differ in one or more ways with respect to the type(s), number, order or modification of the subunits comprising the compounds.

As will be readily apparent to one skilled in the art the present invention is useful for the solid phase synthesis of organic compounds, including peptides.

For the synthesis of compounds, appropriate starting materials may be attached to a combinatorial support. Preferred support materials include solid polymeric materials, such as polyacrylamide, polystyrene, polydextran, polyethylene glycol-grafted polystyrene, cellulose, sephadex resins, acrylics, latex, and combinations thereof. Alternate support materials include glass and ceramics. Synthetic reactions may be conducted on the support-bound starting materials to obtain the desired compounds which may then be cleaved from the combinatorial support.

In a further aspect of the present invention, combinatorial libraries distributed by the methods of the present invention may be screened for pharmacologically or diagnostically useful compounds, as well as for desired physical or chemical properties. It will be clear to one skilled in the art that such screening may be conducted on a library of compounds which are still attached to the support bead, but preferably the screening and other operations are carried out on compounds that have been separated from the combinatorial support.

The present invention is useful for developing new drugs and chemical entities. The invention is useful for rapidly separating and assaying large numbers of molecules that may vary in their chemical structure or composition. The invention is further useful for randomly generating a large number of candidate compounds, then later optimizing those compounds which exhibit the most desirable

properties. The following non-limiting examples are presented to better illustrate the invention.

EXAMPLE 1

Fabrication of Plate, Controlled Distribution of Combinatorial Supports, and Cleavage and Recovery of Compounds

A glass microscope slide (approximately 1 mm thick) is laser drilled with an excimer laser to provide a 5 x 5 square array of holes (each less than about 200 microns in diameter and slightly flared at the top) with about 2 mm between each hole.

A suspension of polystyrene resin beads containing the library of compounds is suspended in a solvent such as dimethyl formamide. The suspension is sprayed or otherwise applied to the plate while a vacuum (for example 5-15 psi) is drawn on the underside of the plate. While maintaining a vacuum on the underside of the plate, excess beads are washed off the plate with solvent to give a controlled distribution of one resin bead per hole.

In a further step, the compounds are cleaved by standard cleavage methodology. A solution containing cleavage reagent may be transfered to the plate by liquid addition, vapor phase transfer or whole plate transfer. Alternatively, the cleavage may be conducted by photolysis. Upon cleavage of the compounds from the beads, the solvent is removed, by a method such as evaporation, and a solvent in which the compounds are soluble (such as dimethyl sulfoxide) is added to each bead. The compounds are solubilized into the solvent and the solution is transfered to a secondary (daughter) plate where the compounds may be assayed or their structure may be determined.

EXAMPLE 2

A full-size (12.0 cm x 7.80 cm) ceramic plate is laser drilled to yield a high density plate having 1536 holes. The plate is 0.50 mm thick with a flatness profile of 50 microns over the surface of the plate. The plate is made of alumina (Catalog No. AD-96 from Coors Ceramics

Co., 1100 Commerce Park Drive, Oak Ridge, Tenessee 37830). The holes on the second (bottom) side of the plate are made with a high intensity carbon dioxide laser, and the holes on the first (top) side of the plate are made with a high powered excimer laser. The openings in both the top (D1) and the bottom (D3) of the plate are about 220 to 240 microns. The diameter of the openings of the holes in the wells (D2) is about 85 microns.

Beads of polystyrene support were distributed onto the plate, with one bead per hole, using the method described above, and using DMF as the liquid in which the beads were suspended. A microscopic examination revealed that virtually every well had one bead on it, and none of the wells had more than on bead.

EXAMPLE 3

A full-size ceramic plate having the dimensions, hole spacings, and hole sizes of Example 2 is made by molding a hydrogel pre-ceramic plate, including holes, followed by high temperature sintering.

While the invention has been described and illustrated with reference to certain particular embodiments thereof, those skilled in the art will appreciate that various adaptations, changes, modifications, substitutions, deletions, or additions of procedures and protocols may be made without departing from the spirit and scope of the invention. For example, reaction conditions other than the particular conditions as set forth herein above may be applicable as a consequence of variations in the reagents or methodology to prepare the compounds from the processes of the invention indicated above. Likewise, the specific reactivity of starting materials may vary according to and depending upon the particular substituents present or the conditions of manufacture, and such expected variations or differences in the results are contemplated in accordance with the objects and practices of the present invention. It is intended, therefore, that the invention be defined by the scope of the claims which follow and that such claims be interpreted as broadly as is reasonable.

CLAIMS:

We claim:

1. A method for distributing combinatorial support particles in a 2-dimensional array on a flat plate, comprising the steps of:

(a) providing: (1) a suspension of combinatorial support particles in a liquid; and (2) a flat plate having a first side and a second side and an array of wells on the first side of the plate, wherein each well has an opening on the first side of the plate, wherein each well is connected to a hole that passes from the well to the second side of the plate, each hole having an opening in the well to which it is connected, and wherein the diameters of substantially all of the combinatorial support particles are larger than the diameters of the openings of the holes in the wells;

(b) applying the suspension of the particles to the first side of the plate;

(c) creating a positive pressure difference between the first side and the second side of the plate by applying a vacuum to the second side of the plate, or a positive pressure to the first side of the plate, or a combination of a vacuum and a positive pressure to the second and first sides of the plate respectively, so that the particles are held in or on the wells by the pressure difference between the first side and the second side of the plate, with one particle being held on or in each well; and

(d) washing the excess particles from the plate with a liquid, so that the particles of support that remain after washing are held on or in the wells, with one particle per well.

2. The method as recited in Claim 1, wherein the particle size is selected such that only one particle fits on or in each well.

3. The method as recited in Claim 2, wherein the particles and wells have an approximately circular cross-section.

4. The method as recited in Claim 3, wherein the particles have a diameter in the range of about 50 microns to about 5,000 microns.

5. The method as recited in Claim 4, wherein the support particles are made from a material selected from the group consisting of solid polymeric materials, glass, and ceramics.

6. A method for distributing combinatorial support beads in a 2-dimensional array on a flat plate, comprising the steps of:

(a) providing: (1) a suspension of combinatorial support beads in a liquid, wherein the beads are approximately spherical in shape and have an average diameter in the range of about 50 microns to about 5,000 microns; and (2) a flat plate having a first side and a second side and a array of wells on the first side, wherein each well has an approximately circular cross section in the plane of the plate with an approximately circular opening on the first side of the plate, wherein each well is connected to a hole that passes from the well to the second side of the plate, each hole having an approximately circular opening in the well to which it is connected, wherein the opening of each hole in the well to which it is connected is smaller than the opening of the well on the first side of the plate and is smaller than substantially all of the beads ;

(b) applying the suspension of beads to the first side of the plate;

(c) creating a positive pressure difference between the first side and the second side of the plate by applying a vacuum to the second side of the plate, or a positive pressure to the first side of the plate, or a combination of a vacuum and a positive pressure to the second and first sides of the plate respectively, so that the beads are held on the openings of the wells by the pressure difference between the first side and the second side of the plate, with one bead being held on the opening of each well; and

(d) washing the excess beads from the plate with a liquid, so that the particles of support that remain after washing are held on the openings of the wells, with one particle per well.

7. The method as recited in Claim 6, wherein the combinatorial beads have an average diameter that is larger than the diameter of the opening at the top of the well.

8. The method as recited in Claim 7, wherein the beads have an average diameter that is larger than the diameter of the opening at the top of the well by a factor of up to about 10.

9. The method as recited in Claim 6, wherein the array of wells are arranged in a square grid having from about 25 holes to about 20,000 holes.

10. The method as recited in Claim 6, wherein the beads are made from a polymer selected from the group consisting of poly(acrylamide), polystyrene, polydextran, polyethylene glycol-grafted polystyrene, cellulose, sephadex resins, acrylics, and latex.

11. A method for distributing combinatorial support beads in a 2-dimensional array on a flat plate, comprising the steps of:

(a) providing: (1) a suspension of combinatorial support beads in a liquid; and (2) a flat plate having a first side and a second side and an array of holes that pass from the first side to the second side of the plate, wherein the holes have openings on the first side and second side of the plate;

(b) applying the suspension of beads to the first side of the plate;

(c) creating a positive pressure difference between the first side and the second side of the plate by applying a vacuum to the second side of the plate, or a positive pressure to the first side of the plate, or a combination of a vacuum and a positive pressure to the second and first sides of the plate respectively, so that the beads are held on the holes by the pressure difference between the first side and the second side of the plate, with one bead being held on the opening of each hole on the first side of the plate; and

(d) washing the excess beads from the plate with a liquid, so that the beads that remain after washing are held on the openings of the holes on the first side of the plate, with one bead per hole.

12. The method as recited in Claim 11, wherein the diameters of substantially all of the beads are larger than the diameters of the openings of the holes on the first side of the plate.

13. The method as recited in Claim 12, wherein the beads and the openings of the holes on the first side of the plate have an approximately circular cross-section, and wherein the beads have an average diameter that is larger than the diameters of the openings of the holes on the first side of the plate by a factor of up to about 10.

14. The method as recited in Claim 13, wherein the beads have an average diameter in the range of about 50 microns to about 5,000 microns.

15. The method as recited in Claim 13, wherein the beads have an average diameter in the range of about 100 microns to about 2,000 microns.

16. The method as recited in Claim 11, wherein the array of holes are arranged in a square grid having from about 25 holes to about 20,000 holes.

17. The method as recited in Claim 11, wherein the support beads are made from a polymer selected from the group consisting of poly(acrylamide), polystyrene, polydextran, polyethylene glycol-grafted polystyrene, cellulose, sephadex resins, acrylics, and latex.

18. A flat plate for use in combinatorial chemistry comprising a first side and a second side and an array of wells on the first side, wherein each well has an approximately circular cross section in the plane of the plate and an approximately circular opening on the first side of the plate, wherein each well is connected to a hole that passes from the well to the second side of the plate, each hole having an approximately circular opening in the well to which it is connected, wherein the opening of each hole in each well is smaller than the opening of the well on the first side of the plate.

19. The flat plate as recited in Claim 18, wherein the diameters of the openings of the wells on the first side of the plate are in the range of about 40 microns to about 4000 microns and the thickness of the plate is in the range of about 0.1 mm to about 5 mm.

20. The flat plate as recited in Claim 19, wherein the plate is made from a material selected from the group consisting of plain glass, derivatized glass, silanized glass, glass having absorbed bio- and non-biopolymers, plastics, metal oxides, metals, and ceramics.

21. The flat plate as recited in Claim 19, wherein the plate is made from a ceramic material.

22. A flat plate for use in combinatorial chemistry comprising a first side and a second side and an array of holes connecting the first side to the second side, wherein each hole has a circular opening on the first side of the plate, the circular opening having a diameter in the range of about 40 microns to about 4000 microns, wherein the thickness of the plate is in the range of about 0.1 mm to about 5 mm.

23. The flat plate as recited in Claim 22, wherein the plate is made from a material selected from the group consisting of plain glass, derivatized glass, silanized glass, glass having absorbed bio- and non-biopolymers, plastics, metal oxides, metals, and ceramics.

24. The flat plate as recited in Claim 22, wherein the plate is made from a ceramic material.

25. An apparatus for distributing combinatorial support particles that are spatially separated on a plate comprising (1) a plate as recited in Claim 19 and (2) means for creating a pressure differential between the first side of the plate and the second side of the plate.

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Application No: GB 9908411.3 **Examiner:** Michael R. Wendt
Claims searched: All **Date of search:** 23 August 1999

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.Q): G1B (BCC, BCB, BCK)

Int Cl (Ed.6): C07K 1/04; B01L 3/00; B01J 19/00

Other: Online: EPODOC, WPI, Japio

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
P,X	WO 98/17382 A1	(GLAXO) e.g. see page 3 lines 14 - 34; page 9 paragraph 1 etc. Claim 1.	1, 6 , 8, 18 & 22 at least
X	WO 98/08092 A1	(SMITHKLINE) e.g. see page 10 lines 16 - 20; page 5 lines 20 etc; tray 10 in Figure 1.	22 at least
X	WO 97/15394 A1	(SMITHKLINE) e.g. see Claim 1 and Figures 1 & 1A.	18 at least.

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
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